UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY AND POLLUTION
PREVENTION

MEMORANDUM

DATE: 4/28/16

SUBJECT: Fluensulfone Dermal Penetration Data Evaluation Record.

PC Code: 050410
Decision No.: 508627
Petition No.: N/A

Risk Assessment Type: N/A
TXR No.: 0057254
MRID No.: 49700504

DP Barcode: D429722
Registration Nos.: 66222-243
Regulatory Action: Product Registration-Section 3
Case No.: N/A
CAS No.: 318290-98-1
40 CFR: §180.680

FROM: Jaime D'Agostino, Toxicologist
Risk Assessment Branch II
Health Effects Division (7509 P)

THRU: Christina Swartz, Chief
Risk Assessment Branch II
Health Effects Division (7509 P)

TO: Mark Suarez
Invertebrate-Vertebrate Branch 3
Registration Division (7505P)

I. CONCLUSION: Risk Assessment Branch II (RAB II) prepared a final data evaluation record of the *in vitro* dermal penetration toxicity study (MRID 49700504) submitted to refine the dermal absorption factor (DAF) of fluensulfone.

II. ACTION REQUESTED: Risk Assessment Branch II (RABII) is currently evaluating proposed new uses for the EC formulation of fluensulfone on multiple row crops and as Nimitz 1.5 GR on root crops. A risk assessment is being prepared as part of that action. In addition, RABII recently evaluated new uses for Nimitz 1.5 GR on turf, for which HED prepared a risk assessment (D424168, Michael Doherty, 1/06/2016). As part of the process, the Health Effects

Division (HED) was asked to provide secondary review and prepare a final data evaluation record for the *in vitro* dermal penetration study submitted by the registrant to refine the DAF.

III. BACKGROUND: Fluensulfone is the first nematicide in the fluoroalkenyl class. Fluensulfone was recently registered for use on cucurbit and fruiting vegetables as an emulsifiable concentrate (EC) formulation (D403767, Michael Doherty, 6/18/2014).

The registrant, ADAMA, submitted new *in vitro* dermal penetration study as part of the proposed new uses on turf and crops. The toxicity studies were reviewed by HED and the results were incorporated into the last risk assessment on turf (D424168, Michael Doherty, 1/06/2016). After reviewing the studies, RAB II prepared a final data evaluation record reflecting HEDs' conclusions with respect to the overall findings as well as the acceptability of the study.

DATA EVALUATION RECORD

FLUENSULFONE

STUDY TYPE: *IN VITRO* DERMAL PENETRATION
OECD 428

MRID 49700504

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task Order No. 6-152

Primary Reviewer: Jennifer L. Rayner, Ph.D., DABT	Signature: Date:	Jennifer L Rayner JLR 09/30/2015
Secondary Reviewers: T. Borges, Ph.D., MT(ASCP), DABT (1994-2014).	Signature: Date:	T. Borges TB 09/30/2015
Robert H. Ross, M.S., Program Manager	Signature: Date:	Robert H. Ross RHR 09/30/2015
Quality Assurance: Angela M. Edmonds, B.S.	Signature: Date:	Angela M Edmonds 09/30/2015

Disclaimer
This review may have been altered subsequent to the contractor's signatures above.

Summitec Corp. for the U.S. Environmental Protection Agency under Contract No.EP-W-11-014

EPA Reviewer: Jaime D'Agostino **Signature:**
Health Effects Division (7509P) **Date:** 4/28/16
EPA Secondary Reviewer: Karlyn Middleton **Signature:**
Health Effects Division (7509P) **Date:** 4/28/16
Template version 09/11

TXR#: 0057254

DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Dermal Penetration Study – Human and Rat
OECD 428

PC CODE: 050410 **DP BARCODE:** Not provided

TEST MATERIAL (PURITY): MCW-2 Technical (Fluensulfone, 96.54% a.i.)

SYNONYMS: 5-Chloro-2-[3,4,4-trifluoro-but-3-ene-1-sulfonyl]-thiazole

CITATION: Bernard, F. (2015) MCW-2: Percutaneous penetration of [^{14}C]-MCW-2 formulated as MCW-2 480 EC through rat and human skin membranes (*in vitro*). Harlan Laboratories Ltd. (Switzerland). Study number D84144, March 10, 2015. MRID 49700504. Unpublished

SPONSOR: ADAMA Makhteshim Ltd., PO Box 60, Beer-Sheva 84100, Israel.

EXECUTIVE SUMMARY: In an *in vitro* dermal penetration study (MRID 49700504), MCW-2 ([unlabeled] 96.54%, Batch No. 36372130-291-PF1; [radiolabeled] 97.55%, Batch No. XX-001 radiolabeled on thiazole-2-^{14}C) was applied to human and rat split thickness skin membrane samples previously placed in automated flow-through diffusion cells (exposure area 0.64 cm^2) at dose levels of 480 g/L (commercial formulation) and 10 g/L (1:50 dilution). Exposure duration was 8 hours and the experiment ended 24 hours after exposure. Physiological saline or ethanol/water (1:1) were used as the perfusates.

For human skin membranes, the penetration of the test item was lower at the low and high dose compared to rat skin membranes. Most, 88.93% of the low dose and 96.63% of the high dose, of the applied dose was dislodged (skin and donor cell washes) in human skin samples when saline was used for the perfusate. In the ethanol/water (1:1) perfusate samples, 71.66% of the low dose and 95.43% of the high dose were dislodged. In rat skin membranes in which saline was used as the perfusate, 53.01% of the low dose and 67.10% of the high dose remained in the skin and donor cell washes. With the use of ethanol/water (1:1), 21.61% of the low dose and 35.52% of the high dose were dislodged. The total absorbed dose was 42.08% and 18.57% (low and high dose), and 6.16% and 0.78% (low and high dose) for rat and human skin membranes, respectively, when using physiological saline as perfusate. The total absorbed dose was 73.66% and 52.12% (low and high dose), and 18.36% and 1.38% (low and high dose) for rat and human skin membranes, respectively, when using ethanol/water (1:1) as perfusate.

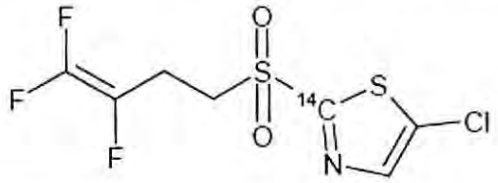
This study in the rat is considered **Acceptable / Non-guideline**. The does not satisfy the EPA guideline requirement for a dermal penetration study (OCSPP 870.7600) in the rat; however, the study does satisfy the standards of OECD 428 for *in vitro* dermal studies under which the study was conducted.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: MCW-2 Technical

Description:	Yellow crystalline solid
Lot/batch #:	36372130-291-PF1 unlabeled; XX-001 radiolabeled
Purity:	96.54% a.i (w/w) unlabeled (verified by HPLC)
Compound stability:	Stored under refrigeration in dark and dry conditions; expiration date February 28, 2016
CAS # for TGAI:	318290-98-1
Structure:	
Vehicle/Solvent used:	Acetonitrile
Radiolabeling:	Fluensufone, [thiazole-2-^{14}C]-
Specific Activity:	2142 MBq/mmol; 7344 kBq/mg
Radiochemical Purity:	97.5% radiochemical purity
Source:	Makhteshim Chemical Works Ltd (sponsor)

Test material: MCW-2 480 EC Blank

Description:	Not available
Lot/batch #:	2354-220414-01
Purity:	0%
Compound stability:	Expiration date April 21, 2016
CAS # for TGAI:	Not available
Vehicle/Solvent used:	Not available
Source:	Makhteshim Chemical Works Ltd

2. **Relevance of test material to proposed formulation(s):** The test preparation was tested at two target concentrations of MCW-2: 480 g/L (commercial formulation) and 10 g/L (1:50 in-use dilution). The concentrate represents the maximal concentration possible when handling the undiluted formulation, and the dilution represents the recommended in-use spray concentration.

3. Skin samples:

Species:	Human
Sex:	3 Males age 30-82 years ; 6 Females age 27-45
Sites:	Abdomen (9)
Source:	Capital Biosciences Inc., Rockville, MD, USA
Strain/Species:	HanRcc: WIST (SPF) rat
Sex:	Males
Age/Weight:	9 weeks; weight not reported
Source:	Harlan Laboratories Ltd.

B. STUDY DESIGN:

1. Dose:

Rationale: The study was designed to examine the *in vitro* rate and extent of dermal absorption of MCW-2 at 480 and 10 g/L in human and rat skin membranes after 8 hours exposure.

Nominal doses: 480 and 10 g/L.

Actual doses: The actual mean doses and radioactivity of test materials applied are listed in Table 1.

Table 1. Mean actual concentrations

Test preparation	Target (g/L)	Applied dose (µg/cm²)	Applied dose (dpm/cell)	Concentration (mg/cm³)
1	480	4547	1,633,624	485
2	10	90.2	527,358	9.6

From page 26 of MRID 49700504.

Dose volume: 6 µL of dose formulation to 0.64 cm^2 application area

Duration of exposures (time from dose to skin wash): 8 hours

Termination periods (time from dose to collection): 8 and 24 hours

Number of skins/group: Saline perfusate: 7 human and 7 rat skins/group; Ethanol/water (1:1): 4 human and 4 rat skins/group

2. Skin preparation: Split thickness skin membranes were prepared from the full-thickness skin membranes. Subcutaneous fat was removed from full thickness skin and 400 µm thick sections were cut using a dermatome. Skin membrane integrity was assessed by applying 50 µl tritiated water to the skin membrane and assessing cumulative penetration over 6 hours. Rat skin membranes with Kp > 3.5 $x10^{-3}$ cm/h and human membranes with Kp > 3.0 $x10^{-3}$ cm/h were not used in the study. The split thickness skin membranes were placed in an automated flow-through diffusion cell apparatus, and the surface area of the exposed skin in the donor chamber was 0.64 cm^2. The cells were was connected to multi-channel peristaltic

pumps, and the effluent was collected into vials on a fraction collector. The cells were heated to 32 ± 1°C and the flow rate of the perfusate was ~3 mL/h.

3. **Dose preparation, administration, and quantification:**

Preparation: Dose formulation preparations are described below. Radiolabeled MCW-2 was supplied as a solution in acetonitrile which was used as the stock solution in the formulations. The solubility in physiological saline and ethanol/water (1:1) was calculated to be 426 and 974 µg/mL, respectively. Homogeneity of the formulations was determined prior to the first, in the middle and after the last administration, and the CV was 2.5% and 0.5% for low and high doses, respectively. Stability of formulations was checked by HPLC at the time of application and was 94.7% and 95.4% for the low and high doses, respectively.

Commercial formulation: Stock solution (1.8 mg, 7344 kBq/mg) was added to unlabeled MCW-2 (1437.9 mg). Acetonitrile was evaporated to dryness under nitrogen. An appropriate volume of formulation blank (2160.0 mg) was added. The final concentration was approximately 467 mg [^{14}C]-MCW-2/mL (4370 kBq/mL).

1:50 in-use dilution: Stock solution (2.1 mg, 7344 kBq/mg) was added to unlabeled MCW-2 (98.1 mg). Acetonitrile was evaporated to dryness under nitrogen. An appropriate volume of formulation blank (150.8 mg). The final concentration was approximately 10 mg [^{14}C]-MCW-2/mL (1502 kBq/mL).

Application: The prepared formulations (6 µL) were applied to the surface of the stratum corneum of samples of human and rat skin membranes with a positive displacement pipette. Permeable tape was used to cover the donor chambers after application. Radioactivity of the applied amount was determined using three controls from each concentration group.

4. **Skin wash:** After 8 hours of exposure, the surfaces of all skin samples were rinsed 3 times with 0.5 mL of a 1% solution of mild shower gel in tap water and brushed twice with cotton swabs (moistened with mild shower gel). The skin was rinsed with 0.5 mL water and dried with one cotton swab. The wash, rinse, and cotton swabs were pooled into a single vial and analyzed. The skin sample was covered with permeable tape until the 24-hour termination period, and washed as described above. The skin wash and swabs were pooled for analysis.

5. **Sample collection:** The perfusate was collected every hour from 0 to 8 hours post dose and every two hours from 8 to 24 hours post dose. After the 24-hour skin wash, the skin was stripped with 15 tape strips that were retained and analyzed. Tape strips 1-5 were analyzed individually and tape strips 6-10 and 11-15 were pooled for analysis. The donor cell was washed with 15 mL of ethanol/water (1:1) which was analyzed.

6. **Sample preparation and analysis:** Details of sample preparation are provided in Table 2. Samples were analyzed after sampling or kept refrigerated until analysis.

Table 2. Sample preparation details	
Media	**Preparation**
Perfusate, skin membrane wash (wash+ cotton swabs), donor cell wash	Samples were mixed with scintillation fluid and the radioactivity measured by LSC.
Tape strips, skin membranes	Samples were dissolved in Solvable® digest, added to scintillation fluid, and the radioactivity determined by LSC.

7. Analytical methods:

LSC - Radioactivity in all samples (single sample per measurement) was measured by LSC on a Packard Tri-Carb 2500TR liquid scintillation counter equipped for computing quench-correcting disintegrations per minute (DPM). Blank sample (scintillation mixture only) values were subtracted from sample count rates to provide net DPM/sample.

HPLC - Stability (radioactive purity) of the test item in the formulation was measured by HPLC. The radioactive signal of the applied test item was monitored using a radioactivity flow monitor.

II. RESULTS:

A. SUMMARY TABLES:

Table 3 shows the mean radioactivity distribution with the two treatment groups; the detailed [^{14}C]-MCW-2 distribution for individual cells and means are presented Appendix A (Tables A1-A8). Summary data are presented in Appendix B (Tables B).

B. TOTAL ABSORBED DOSE:

Results of analysis are summarized in Table 3. There was a clear difference in the penetration of [^{14}C]-MCW-2 between physiological saline and ethanol:water (1:1 v/v). When ethanol:water (1:1 v/v) was used as perfusate, the flux increased approximately 2-fold and 3-fold at the low and high dose, respectively for both species compared to when physiological saline was used.

Human

After application of [^{14}C]-MCW-2, the penetration of radioactivity through the skin membrane accounted for 3.84% and 0.13% within 8 hours for the low and high dose, respectively, when saline was used as the perfusate. Within 24 hours an average of 5.75% of the low dose and 0.52% of the high dose penetrated through the rat skin membrane into the perfusate. The flux, which reflects the penetration rate under steady-state conditions, was calculated to be 1.171 µg/cm^2/h for the low dose level and 0.929 µg/cm^2/h for the high dose level. At the end of the 8-hour exposure period, radioactivity was removed from the application site by skin membrane wash, accounting for 74.43% (low dose) and 94.63% (high dose). An additional amount of radioactivity, 13.56% and 1.23% was removed by the second skin membrane wash at 24 hours. In total, 88.93% of the low dose and 96.63% of the high dose was recovered in skin membrane wash at 8 and 24 hours and in donor cell wash. The first two tape strips removed 2.52% of the low dose and 0.20% of the high dose. A further 0.83% and 0.24% of the applied low and high dose, respectively, were removed with

additional tape strips. After tape stripping, 0.41%, and 0.26% remained in the skin membrane for the low and high dose level, respectively. The total absorption was calculated to be 6.16% and 0.78% for the low and high dose level, respectively.

After application of [^{14}C]-MCW-2, the penetration of radioactivity through the skin membrane accounted for 10.50% and 0.41% within 8 hours for the low and high dose, respectively, when ethanol/water (1:1) was used as the perfusate. Within 24 hours an average of 17.80% of the low dose and 1.23% of the high dose penetrated through the rat skin membrane into the perfusate. The flux, which reflects the penetration rate under steady-state conditions, was calculated to be 2.636 µg/cm^2/h for the low dose level and 2.673 µg/cm^2/h for the high dose level. At the end of the 8-hour exposure period, radioactivity was removed from the application site by skin membrane wash, accounting for 55.85% (low dose) and 93.46% (high dose). An additional amount of 12.91% and 1.65% was removed by the second skin membrane wash at 24 hours. In total, 71.66% of the low dose and 95.43% of the high dose was recovered in skin membrane wash at 8 and 24 hours and in donor cell wash. The first two tape strips removed 6.55% of the low dose and 0.43% of the high dose. A further 1.60% and 0.18% of the applied low and high dose, respectively, were removed with additional tape strips. After tape stripping, 0.56%, and 0.15% remained in the skin membrane for the low and high dose level, respectively. The total absorption was calculated to be 18.36% and 1.38% for the low and high dose level, respectively.

Rat

After application of [^{14}C]-MCW-2, the penetration of radioactivity through the skin membrane accounted for 27.97% and 6.81% within 8 hours for the low and high dose, respectively, when saline was used as the perfusate. Within 24 hours an average of 40.63% of the low dose and 13.47% of the high dose penetrated through the rat skin membrane into the saline perfusate. The flux, which reflects the penetration rate under steady-state conditions, was calculated to be 3.886 µg/cm^2/h for the low dose level and 46.088 µg/cm^2/h for the high dose level. At the end of the 8-hour exposure period, radioactivity was removed by skin membrane wash, accounting for 51.04% (low dose) and 64.01% (high dose). An additional amount of radioactivity, 1.97% and 1.77%, was removed by the second skin membrane wash at 24 hours. In total, 53.01% of the low dose and 67.10% of the high dose was recovered in skin membrane wash at 8 and 24 hours and in donor cell wash. The first two tape strips removed 3.38% of the low dose and 3.80% of the high dose. A further 1.59% and 4.12% of the applied low and high dose, respectively, were removed with additional tape strips. After tape stripping, 1.45%, and 5.10% remained in the skin membrane for the low and high dose level, respectively. The total absorption was calculated to be 42.08% and 18.57% for the low and high dose level, respectively.

After application of [^{14}C]-MCW-2, the penetration of radioactivity through the skin membrane accounted for 52.35% and 21.05% within 8 hours for the low and high dose, respectively, when ethanol/water (1:1) was used as the perfusate. Within 24 hours an average of 67.87% of the low dose and 36.06% of the high dose penetrated through the rat skin membrane into the perfusate. The flux, which reflects the penetration rate under steady-state conditions, was calculated to be 7.972 µg/cm^2/h for the low dose level and 139.625 µg/cm^2/h for the high dose level. At the end of the 8-hour exposure period, radioactivity was removed from the application site by skin membrane wash, accounting for 20.94% (low dose) and 33.35% (high dose). An additional amount of radioactivity, 0.64% and 1.24% was

removed by the second skin membrane wash at 24 hours. In total, 21.61% of the low dose and 35.52% of the high dose was recovered in skin membrane wash at 8 and 24 hours and in donor cell wash. The first two tape strips removed 0.09% of the low dose and 1.62% of the high dose. A further 0.39% and 4.80% of the applied low and high dose, respectively, were removed with additional tape strips. After tape stripping, 5.79%, and 16.06% remained in the skin membrane for the low and high dose level, respectively. The total absorption was calculated to be 73.66% and 52.12% for the low and high dose level, respectively.

Table 3. Recovery (% applied dose)

Compartment	Formulations			
	Human		Rat	
	10 g/L	480 g/L	10 g/L	480 g/L
Saline perfusate				
Dislodged dose	88.93	96.63	53.01	67.10
Total unabsorbed dose*	92.28	97.07	57.98	75.02
Tape strips 1-2	2.52	0.20	3.38	3.80
Tape strips 3-15	0.83	0.24	1.59	4.12
Absorbed dose	6.16	0.78	42.08	18.57
Perfusate	5.75	0.52	40.63	13.47
Skin membrane	0.41	0.26	1.45	5.10
Potentially absorbable dose*	7.4	1.28	45.12	27.79
Recovery	98.44	97.85	100.06	93.59
Ethanol/water (1:1) perfusate				
Dislodged dose	71.66	95.43	21.61	53.52
Total unabsorbed dose*	79.81	96.04	22.09	59.95
Tape strips 1-2	6.55	0.43	0.09	1.63
Tape strips 3-15	1.60	0.18	0.39	4.80
Absorbed dose	18.36	1.38	73.66	52.12
Perfusate	17.8	1.23	67.87	36.06
Skin membrane	0.56	0.15	5.79	16.06
Potentially absorbable dose*	20.52	1.71	79.84	72.98
Recovery	98.19	97.41	95.75	94.07

Data extracted from Summary Table (p. 12) and Tables 25-32, pp. 59-66, MRID 49700504.
Total unabsorbed dose = dislodged dose + stratum corneum tape strips 1-15
Skin membrane = dose remaining in the skin membrane
Absorbed dose = skin membrane + perfusates
Potentially absorbable dose= absorbed dose + skin membrane + stratum corneum tape strips 3-15
* Calculated by the reviewer

C. POTENTIALLY ABSORBABLE DOSE:

The reviewer calculated the potential absorbable dose based on values provided in Appendix A. The mean potential absorbable doses are shown in Table 3.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The total absorption of MCW-2 formulated as MCW-2 480 EC through rat and human skin membrane, calculated as dose in perfusate and stripped skin membrane, was high in rat skin for both doses and moderate (low dose) and low (high dose) in human skin. The total absorption was calculated to be 42.08% and 18.57% (low and high dose), and 6.16% and

0.78% (low and high dose) for rat and human skin membranes, respectively when using physiological saline as perfusate. The total absorption was calculated to be 73.66% and 52.12% (low and high dose), and 18.36% and 1.38% (low and high dose) for rat and human skin membranes, respectively when using ethanol/water (1:1) as perfusate. The use of solvent (ethanol:water 1:1 v/v) in the perfusate increased (approximately 2-3 fold) the total absorption compare to when physiological saline NaCl 0.9% is used as perfusate. Human skin membranes were significantly less permeable compared to rat skin membranes.

B. REVIEWER COMMENTS:

The Reviewer agrees with the Study Author's assessment. In this study, human and rat split thickness skin membrane samples were dermally exposed to MCW-2 as the commercial formulation (480 g/L) or 1:50 dilution (10 g/L) for 8 hours. Radioactivity of the skin samples were taken at 8 and 24 hours. Perfusate (physiological saline or ethanol/water [1:1]), skin wash, and rinse were retained and analyzed. In addition, 15 tape strips were collected and analyzed at the end of the experiment.

The total absorbed dose was 42.08% and 18.57% (low and high dose), and 6.16% and 0.78% (low and high dose) for rat and human skin membranes, respectively, when using physiological saline as perfusate. The total absorbed dose was 73.66% and 52.12% (low and high dose), and 18.36% and 1.38% (low and high dose) for rat and human skin membranes, respectively, when using ethanol/water (1:1) as perfusate. The reviewer agrees that ethanol/water (1:1) increased absorption in both species and that human skin appeared to be less permeable than rat skin.

C. STUDY DEFICIENCIES:

No study deficiencies were noted. Therefore, the Reviewer considers the study **Acceptable / Non-guideline**.

D. REFERENCES:

References used to evaluate this study included the following:

OECD Environmental Health and Safety Publications, Series on Testing and Assessment No. 28. Guidance document for the conduct of skin absorption studies (Paris, March 2004).

OECD guideline for the testing of chemicals. Guideline No. 428; Skin Absorption: *in vitro* Method (Paris, April 2004).

OECD Principles of Good Laboratory Practice (as revised in 1997), Paris, ENV/MC/CHEM (98) 17.

Appendix A

Table A1. Dis Distribution of radioactivity (% of applied dose) of ^{14}C-MCW-2 in test preparation 1 (10 g/L, 1:50 dilution with saline perfusate) to human skin

[% of Dose]									
Dose applied [µg·cm^{-2}]	90.2								
Cell No.	Cell 8	Cell 9	Cell 10	Cell 11	Cell 12	Cell 13	Cell 14	**Mean**	SD
Dislodged Dose									
Membrane Wash 8 h	70.60	72.84	69.81	68.36	62.87	88.23	88.27	**74.43**	9.92
Membrane Wash 24 h	14.15	15.79	19.18	17.86	21.56	2.94	3.41	**13.56**	7.47
Donor-Cellwash	0.55	1.33	1.22	1.03	1.68	0.54	0.31	**0.95**	0.50
Subtotal	85.30	89.96	90.21	87.26	86.12	91.70	92.00	**88.93**	2.70
Perfusates									
0- 8 h	5.89	3.15	2.16	2.74	1.61	6.09	5.24	**3.84**	1.86
8-12 h	1.09	0.99	0.98	1.02	0.86	1.02	1.03	**1.00**	0.07
12-24 h	0.98	1.13	1.00	1.06	1.42	0.42	0.39	**0.92**	0.38
Subtotal	7.96	5.27	4.14	4.82	3.89	7.53	6.67	**5.75**	1.64
Remaining Dose									
Tape Strips I	1.26	1.59	1.03	3.62	6.71	0.13	0.06	**2.06**	2.37
Tape Strips II	0.34	0.76	0.43	1.03	0.60	0.07	0.03	**0.46**	0.36
Subtotal	1.60	2.35	1.46	4.64	7.31	0.20	0.09	**2.52**	2.61
Tape Strips III	0.29	0.40	0.19	0.33	0.35	0.03	< 0.01	**0.23**	0.16
Tape Strips IV	0.22	1.16	0.08	0.20	0.24	0.03	< 0.01	**0.28**	0.40
Tape Strips V	0.16	0.02	0.08	0.11	0.13	0.03	< 0.01	**0.08**	0.06
Tape Strips VI	0.82	0.05	0.27	0.15	0.21	0.03	0.07	**0.23**	0.28
Tape Strips VII	< 0.01	< 0.01	< 0.01	0.01	0.06	0.02	< 0.01	**0.02**	0.02
Subtotal	1.50	1.63	0.62	0.80	0.99	0.15	0.09	**0.83**	0.60
Skin Membrane	0.28	0.32	0.26	0.64	1.19	0.12	0.05	**0.41**	0.39
Recovery	**96.64**	**99.53**	**96.68**	**98.16**	**99.49**	**99.70**	**98.90**	**98.44**	1.32

Tape Strip I - V individual 1st -5th tape strip
Tape Strip VI pooled tape strip 6-10
Tape Strip VII pooled tape strip 11-15
Subtotal, total, mean, and SD values were calculated using the actual individual values with all digits

From p. 61 of MRID 49700504.

Table A2. Distribution of radioactivity (% of applied dose) of ^{14}C-MCW-2 in test preparation 1 (10 g/L, 1:50 dilution with ethanol/water [1:1] perfusate) to human skin

[% of Dose]						
Dose applied [µg·cm^{-2}]	90.2					
Cell No.	Cell 22	Cell 24	Cell 25	Cell 26	**Mean**	SD
Dislodged Dose						
Membrane Wash 8 h	51.55	53.62	54.56	63.66	**55.85**	5.36
Membrane Wash 24 h	16.66	12.15	12.17	10.64	**12.91**	2.60
Donor-Cellwash	0.89	0.84	1.07	8.83	**2.91**	3.95
Subtotal	69.10	66.61	67.80	83.13	**71.66**	7.71
Perfusates						
0- 8 h	8.78	11.29	15.27	6.68	**10.50**	3.69
8-12 h	2.67	6.69	3.68	2.04	**3.77**	2.06
12-24 h	3.76	5.24	3.68	1.43	**3.53**	1.57
Subtotal	15.22	23.21	22.63	10.15	**17.80**	6.27
Remaining Dose						
Tape Strips I	7.99	4.76	4.57	2.25	**4.89**	2.36
Tape Strips II	1.91	1.70	1.57	1.47	**1.66**	0.19
Subtotal	9.90	6.46	6.14	3.72	**6.55**	2.54
Tape Strips III	0.88	0.76	0.66	0.38	**0.67**	0.21
Tape Strips IV	0.34	0.22	0.21	0.11	**0.22**	0.10
Tape Strips V	0.23	0.11	0.11	0.06	**0.13**	0.07
Tape Strips VI	1.28	0.27	0.29	0.29	**0.53**	0.50
Tape Strips VII	< 0.01	0.10	0.05	0.04	**0.05**	0.04
Subtotal	2.74	1.46	1.33	0.88	**1.60**	0.80
Skin Membrane	0.52	0.60	0.77	0.36	**0.56**	0.17
Recovery	**97.48**	**98.35**	**98.67**	**98.24**	**98.19**	0.50

Tape Strip I - V individual 1st -5th tape strip
Tape Strip VI pooled tape strip 6-10
Tape Strip VII pooled tape strip 11-15
Subtotal, total, mean, and SD values were calculated using the actual individual values with all digits

From p. 62 of MRID 49700504.

Table A3. Distribution of radioactivity (% of applied dose) of ^{14}C-MCW-2 in test preparation 1 (10 g/L, 1:50 dilution with saline perfusate) to rat skin

[% of Dose]									
Dose applied [µg·cm^{-2}]	90.2								
Cell No.	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	**Mean**	SD
Dislodged Dose									
Membrane Wash 8 h	51.75	53.52	48.99	57.12	48.96	48.20	48.72	**51.04**	3.31
Membrane Wash 24 h	2.29	1.92	1.81	1.72	1.34	1.37	3.35	**1.97**	0.69
Donor-Cellwash	0.03	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	**< 0.01**	0.01
Subtotal	54.07	55.44	50.81	58.84	50.30	49.57	52.08	**53.01**	3.31
Perfusates									
0- 8 h	26.17	28.77	31.01	24.47	29.91	30.08	25.36	**27.97**	2.59
8-12 h	8.06	7.80	8.72	6.80	8.63	9.34	9.90	**8.46**	1.03
12-24 h	4.46	4.91	2.84	3.23	2.92	3.90	7.09	**4.20**	1.50
Subtotal	38.69	41.49	42.58	34.50	41.45	43.32	42.36	**40.63**	3.08
Remaining Dose									
Tape Strips I	0.35	2.96	5.98	0.28	5.51	0.66	4.25	**2.86**	2.47
Tape Strips II	0.04	0.66	1.28	0.33	0.23	0.47	0.66	**0.52**	0.40
Subtotal	0.39	3.62	7.27	0.60	5.74	1.13	4.91	**3.38**	2.73
Tape Strips III	0.02	0.31	0.16	4.30	0.14	3.68	0.05	**1.24**	1.89
Tape Strips IV	0.01	0.09	0.01	0.03	0.03	0.02	0.08	**0.04**	0.03
Tape Strips V	0.02	0.42	0.03	0.06	0.02	0.14	0.07	**0.11**	0.14
Tape Strips VI	0.12	0.68	0.08	< 0.01	0.04	0.16	0.09	**0.17**	0.23
Tape Strips VII	0.03	0.12	< 0.01	0.02	< 0.01	0.03	0.05	**0.04**	0.04
Subtotal	0.20	1.63	0.28	4.42	0.22	4.02	0.33	**1.59**	1.87
Skin Membrane	6.48	1.17	0.42	0.39	0.31	0.69	0.70	**1.45**	2.24
Recovery	**99.82**	**103.35**	**101.35**	**98.74**	**98.02**	**98.73**	**100.37**	**100.06**	1.84

Tape Strip I - V individual 1st -5th tape strip
Tape Strip VI pooled tape strip 6-10
Tape Strip VII pooled tape strip 11-15
Subtotal, total, mean, and SD values were calculated using the actual individual values with all digits

From p. 59 of MRID 49700504.

Table A4. Distribution of radioactivity (% of applied dose) of ^{14}C-MCW-2 in test preparation 1 (10 g/L, 1:50 dilution with ethanol/water [1:1] perfusate) to rat skin.

[% of Dose]						
Dose applied [µg·cm^{-2}]	90.2					
Cell No.	Cell 15	Cell 16	Cell 17	Cell 18	**Mean**	SD
Dislodged Dose						
Membrane Wash 8 h	15.20	18.04	22.50	28.01	**20.94**	5.59
Membrane Wash 24 h	0.62	0.76	0.66	0.54	**0.64**	0.09
Donor-Cellwash	0.05	< 0.01	0.02	0.02	**0.02**	0.02
Subtotal	15.87	18.80	23.19	28.57	**21.61**	5.53
Perfusates						
0- 8 h	57.42	57.32	57.29	37.39	**52.35**	9.98
8-12 h	12.73	11.30	7.01	13.88	**11.23**	3.00
12-24 h	4.23	5.35	2.97	4.60	**4.29**	0.99
Subtotal	74.38	73.98	67.27	55.87	**67.87**	8.64
Remaining Dose						
Tape Strips I	0.09	< 0.01	0.02	< 0.01	**0.03**	0.04
Tape Strips II	0.11	0.12	< 0.01	< 0.01	**0.06**	0.06
Subtotal	0.20	0.13	0.03	< 0.01	**0.09**	0.09
Tape Strips III	0.03	0.06	0.03	< 0.01	**0.03**	0.02
Tape Strips IV	0.11	0.07	< 0.01	< 0.01	**0.05**	0.05
Tape Strips V	0.07	0.05	0.49	< 0.01	**0.15**	0.22
Tape Strips VI	0.19	0.08	0.19	0.03	**0.12**	0.08
Tape Strips VII	0.06	< 0.01	< 0.01	0.08	**0.04**	0.04
Subtotal	0.46	0.28	0.70	0.13	**0.39**	0.25
Skin Membrane	6.61	5.90	5.08	5.58	**5.79**	0.64
Recovery	**97.52**	**99.08**	**96.26**	**90.15**	**95.75**	3.91

Tape Strip I - V individual 1st -5th tape strip
Tape Strip VI pooled tape strip 6-10
Tape Strip VII pooled tape strip 11-15
Subtotal, total, mean, and SD values were calculated using the actual individual values with all digits

From p. 60 of MRID 49700504.

Table A5. Distribution of radioactivity (% of applied dose) of ^{14}C-MCW-2 in test preparation 1 (480 g/L, concentrate of commercial formulation with saline perfusate) to human skin

[% of Dose]									
Dose applied [µg·cm^{-2}]	4547.4								
Cell No.	Cell 8	Cell 9	Cell 10	Cell 11	Cell 12	Cell 13	Cell 14	**Mean**	SD
Dislodged Dose									
Membrane Wash 8 h	96.09	98.41	97.05	96.45	95.53	84.92	93.95	**94.63**	4.49
Membrane Wash 24 h	0.51	0.24	0.90	0.55	1.18	2.76	2.51	**1.23**	1.00
Donor-Cellwash	0.68	0.01	0.19	0.35	0.33	3.47	0.31	**0.76**	1.21
Subtotal	97.27	98.66	98.14	97.36	97.03	91.15	96.76	**96.63**	2.50
Perfusates									
0- 8 h	0.16	0.22	0.19	0.02	0.09	0.14	0.07	**0.13**	0.07
8-12 h	0.13	0.13	0.16	0.07	0.11	0.70	0.14	**0.21**	0.22
12-24 h	0.06	0.09	0.18	0.19	0.16	0.33	0.26	**0.18**	0.09
Subtotal	0.35	0.45	0.53	0.28	0.35	1.17	0.47	**0.52**	0.30
Remaining Dose									
Tape Strips I	0.04	0.02	0.03	0.09	0.07	0.48	0.12	**0.12**	0.16
Tape Strips II	0.01	0.01	0.02	0.06	0.03	0.43	0.03	**0.08**	0.15
Subtotal	0.05	0.03	0.05	0.15	0.10	0.90	0.15	**0.20**	0.31
Tape Strips III	0.01	< 0.01	< 0.01	0.11	0.01	0.43	0.01	**0.08**	0.16
Tape Strips IV	0.02	< 0.01	< 0.01	0.03	< 0.01	0.19	0.01	**0.04**	0.07
Tape Strips V	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.06	< 0.01	**0.01**	0.02
Tape Strips VI	< 0.01	< 0.01	0.01	0.10	0.01	0.50	< 0.01	**0.09**	0.18
Tape Strips VII	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.10	< 0.01	**0.02**	0.04
Subtotal	0.04	0.01	0.03	0.24	0.04	1.28	0.04	**0.24**	0.46
Skin Membrane	0.01	0.02	0.03	0.40	0.08	1.12	0.19	**0.26**	0.40
Recovery	**97.73**	**99.17**	**98.77**	**98.43**	**97.60**	**95.63**	**97.61**	**97.85**	1.16

Tape Strip I - V individual 1st -5th tape strip
Tape Strip VI pooled tape strip 6-10
Tape Strip VII pooled tape strip 11-15
Subtotal, total, mean, and SD values were calculated using the actual individual values with all digits

From p. 65 of MRID 4700504.

Table A6. Distribution of radioactivity (% of applied dose) of ^{14}C-MCW-2 in test preparation 1 (480 g/L, concentrate of commercial formulation with ethanol/water [1:1] perfusate) to human skin

[% of Dose]						
Dose applied [µg·cm^{-2}]	4547.4					
Cell No.	Cell 22	Cell 23	Cell 24	Cell 27	**Mean**	SD
Dislodged Dose						
Membrane Wash 8 h	93.59	96.36	92.43	91.47	**93.46**	2.12
Membrane Wash 24 h	1.19	0.19	2.61	2.59	**1.65**	1.17
Donor-Cellwash	0.63	0.02	0.49	0.14	**0.32**	0.29
Subtotal	95.41	96.57	95.53	94.19	**95.43**	0.97
Perfusates						
0- 8 h	0.51	0.72	0.20	0.22	**0.41**	0.25
8-12 h	0.66	0.51	0.36	0.28	**0.45**	0.17
12-24 h	0.51	0.34	0.36	0.25	**0.37**	0.11
Subtotal	1.68	1.57	0.91	0.75	**1.23**	0.46
Remaining Dose						
Tape Strips I	0.05	0.02	0.82	0.31	**0.30**	0.37
Tape Strips II	0.04	0.01	0.22	0.24	**0.13**	0.12
Subtotal	0.09	0.03	1.04	0.55	**0.43**	0.47
Tape Strips III	0.02	< 0.01	0.10	0.09	**0.06**	0.05
Tape Strips IV	0.02	< 0.01	0.08	0.03	**0.04**	0.03
Tape Strips V	0.01	< 0.01	0.04	0.01	**0.02**	0.02
Tape Strips VI	0.04	0.02	0.07	0.08	**0.05**	0.03
Tape Strips VII	< 0.01	< 0.01	0.02	0.03	**0.01**	0.01
Subtotal	0.10	0.05	0.32	0.24	**0.18**	0.13
Skin Membrane	0.13	0.08	0.19	0.19	**0.15**	0.06
Recovery	**97.41**	**98.30**	**97.99**	**95.93**	**97.41**	1.05

Tape Strip I - V individual 1st -5th tape strip
Tape Strip VI pooled tape strip 6-10
Tape Strip VII pooled tape strip 11-15
Subtotal, total, mean, and SD values were calculated using the actual individual values with all digits

From p. 66 of MRID 49700504.

Table A7. Distribution of radioactivity (% of applied dose) of ^{14}C-MCW-2 in test preparation 1 (480 g/L, concentrate of commercial formulation with saline perfusate) to rat skin

[% of Dose]									
Dose applied [µg·cm^{-2}]	4547.4								
Cell No.	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	**Mean**	SD
Dislodged Dose									
Membrane Wash 8 h	62.42	68.92	60.93	86.29	34.47	57.36	77.68	**64.01**	16.51
Membrane Wash 24 h	1.33	0.81	1.04	2.13	5.30	0.24	1.56	**1.77**	1.67
Donor-Cellwash	0.48	0.03	0.92	0.18	5.64	1.51	0.42	**1.31**	1.97
Subtotal	64.22	69.76	62.89	88.61	45.42	59.11	79.65	**67.10**	14.07
Perfusates									
0- 8 h	7.49	8.57	9.56	4.68	3.37	8.44	5.54	**6.81**	2.30
8-12 h	3.92	4.36	4.27	1.99	1.42	3.10	2.37	**3.06**	1.17
12-24 h	4.57	4.78	4.58	2.21	3.66	2.82	2.58	**3.60**	1.07
Subtotal	15.99	17.72	18.41	8.88	8.45	14.36	10.49	**13.47**	4.18
Remaining Dose									
Tape Strips I	1.78	0.39	1.00	0.61	10.92	3.57	0.77	**2.72**	3.77
Tape Strips II	0.40	0.20	1.18	0.17	3.14	1.81	0.70	**1.08**	1.08
Subtotal	2.17	0.59	2.18	0.78	14.05	5.38	1.47	**3.80**	4.79
Tape Strips III	0.18	1.01	0.74	0.05	4.21	1.58	0.35	**1.16**	1.45
Tape Strips IV	0.17	0.09	0.28	0.01	2.62	1.06	0.34	**0.65**	0.94
Tape Strips V	0.15	0.06	0.24	0.02	2.12	0.94	0.12	**0.52**	0.77
Tape Strips VI	0.72	0.22	1.62	0.03	5.08	2.76	0.56	**1.57**	1.81
Tape Strips VII	0.05	0.01	0.34	< 0.01	0.62	0.37	0.10	**0.21**	0.24
Subtotal	1.26	1.38	3.22	0.13	14.66	6.71	1.47	**4.12**	5.12
Skin Membrane	5.43	0.44	6.18	0.30	9.24	10.40	3.72	**5.10**	3.94
Recovery	**89.07**	**89.88**	**92.89**	**98.70**	**91.82**	**95.97**	**96.81**	**93.59**	3.65

Tape Strip I - V individual 1st -5th tape strip
Tape Strip VI pooled tape strip 6-10
Tape Strip VII pooled tape strip 11-15
Subtotal, total, mean, and SD values were calculated using the actual individual values with all digits

From p. 63 of MRID 49700504.

Table A8. Distribution of radioactivity (% of applied dose) of ^{14}C-MCW-2 in test preparation 1 (480 g/L, concentrate of commercial formulation with ethanol/water [1:1] perfusate) to rat skin

[% of Dose]						
Dose applied [µg·cm^{-2}]	4547.4					
Cell No.	Cell 15	Cell 16	Cell 17	Cell 18	**Mean**	SD
Dislodged Dose						
Membrane Wash 8 h	27.74	42.19	41.09	22.37	**33.35**	9.84
Membrane Wash 24 h	1.79	0.78	1.84	0.54	**1.24**	0.68
Donor-Cellwash	0.84	0.35	0.57	2.00	**0.94**	0.73
Subtotal	30.37	43.32	43.50	24.90	**35.52**	9.38
Perfusates						
0- 8 h	19.13	22.83	24.26	17.97	**21.05**	2.98
8-12 h	11.30	10.15	6.62	6.19	**8.57**	2.54
12-24 h	11.08	6.10	3.83	4.79	**6.45**	3.22
Subtotal	41.51	39.08	34.70	28.96	**36.06**	5.51
Remaining Dose						
Tape Strips I	1.64	0.47	0.64	0.44	**0.79**	0.57
Tape Strips II	1.63	0.34	0.82	0.51	**0.83**	0.57
Subtotal	3.27	0.81	1.46	0.95	**1.62**	1.13
Tape Strips III	1.80	0.28	0.50	0.65	**0.81**	0.68
Tape Strips IV	1.24	0.16	0.75	0.95	**0.78**	0.46
Tape Strips V	0.70	0.16	0.80	0.57	**0.56**	0.28
Tape Strips VI	3.31	0.70	2.91	2.29	**2.30**	1.15
Tape Strips VII	0.32	0.20	0.41	0.51	**0.36**	0.13
Subtotal	7.37	1.49	5.37	4.98	**4.80**	2.44
Skin Membrane	14.16	13.20	10.38	26.52	**16.06**	7.15
Recovery	**96.68**	**97.90**	**95.41**	**86.31**	**94.07**	5.28

Tape Strip I - V individual 1st -5th tape strip
Tape Strip VI pooled tape strip 6-10
Tape Strip VII pooled tape strip 11-15
Subtotal, total, mean, and SD values were calculated using the actual individual values with all digits

From p. 64 of MRID 49700504.

Appendix B
Table B1. Summary of results (% of applied dose) at to human and rat skin.

Recovery [% of Dose]								
Test System	Rat Skin Membranes				Human Skin Membranes			
Formulation	A1	A1a	A2	A2a	A1	A1a	A2	A2a
Number of cells	7	4	7	4	7	4	7	4
Dose applied [µg·cm^{-2}]	90.2	90.2	4547.4	4547.4	90.2	90.2	4547.4	4547.4
Perfusates								
0- 8 h	27.97	52.35	6.81	21.05	3.84	10.50	0.13	0.41
8-12 h	8.46	11.23	3.06	8.57	1.00	3.77	0.21	0.45
12-24 h	4.20	4.29	3.60	6.45	0.92	3.53	0.18	0.37
Subtotal	40.63	67.87	13.47	36.06	5.75	17.80	0.52	1.23
Skin Membrane	1.45	5.79	5.10	16.06	0.41	0.56	0.26	0.15
Total Absorption	**42.08**	**73.66**	**18.57**	**52.12**	**6.16**	**18.36**	**0.78**	**1.38**
Membrane Wash 8 h	51.04	20.94	64.01	33.35	74.43	55.85	94.63	93.46
Membrane Wash 24 h	1.97	0.64	1.77	1.24	13.56	12.91	1.23	1.65
Donor-Cellwash	< 0.01	0.02	1.31	0.94	0.95	2.91	0.76	0.32
Dislodged Dose	**53.01**	**21.61**	**67.10**	**35.52**	**88.93**	**71.66**	**96.63**	**95.43**
Tape Strips I	2.86	0.03	2.72	0.79	2.06	4.89	0.12	0.30
Tape Strips II	0.52	0.06	1.08	0.83	0.46	1.66	0.08	0.13
Subtotal	3.38	0.09	3.80	1.62	2.52	6.55	0.20	0.43
Tape Strips III	1.24	0.03	1.16	0.81	0.23	0.67	0.08	0.06
Tape Strips IV	0.04	0.05	0.65	0.78	0.28	0.22	0.04	0.04
Tape Strips V	0.11	0.15	0.52	0.56	0.08	0.13	0.01	0.02
Tape Strips VI	0.17	0.12	1.57	2.30	0.23	0.53	0.09	0.05
Tape Strips VII	0.04	0.04	0.21	0.36	0.02	0.05	0.02	0.01
Subtotal	1.59	0.39	4.12	4.80	0.83	1.60	0.24	0.18
Tape Strips	**4.97**	**0.48**	**7.92**	**6.42**	**3.35**	**8.16**	**0.44**	**0.61**
Recovery	**100.06**	**95.75**	**93.59**	**94.07**	**98.44**	**98.19**	**97.85**	**97.41**
Flux [µg/cm^2/h]	**3.886**	**7.972**	**46.09**	**139.6**	**1.171**	**2.636**	**0.929**	**2.673**
Human/Rat ratio based on Flux					**0.30**	**0.33**	**0.02**	**0.02**
Human/Rat ratio based on % Absorption					**0.15**	**0.25**	**0.04**	**0.03**

A1= low dose with saline perfusate
A1a= low dose with ethanol/water (1:1) perfusate
A2= high dose with saline perfusate
A2a= high dose with ethanol/water (1:1) perfusate

From p. 12 of MRID 49700504.